Supplemental Information

A non-lymphoid origin for lymph node resident memory T cells

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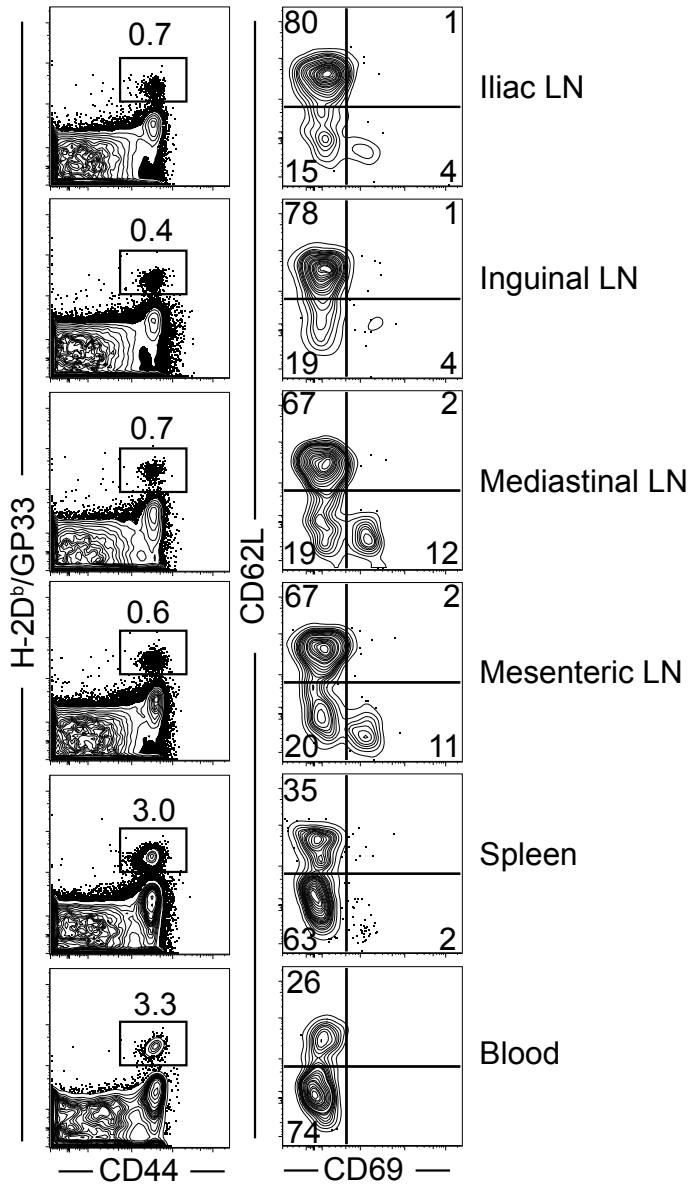

Figure S1. Related to figure 2. Phenotypic analysis of endogenous gp33-specific SLO Trm cells. C57Bl/6 mice were infected with 2X10^5 PFU of LCMV-Armstrong i.p. Indicated LNs, spleen and blood was harvested 90 days after infection. The Gp33-specific CD8$^+$ T cells were identified using a MHC I tetramer (H-2D^b/GP33) and are shown in the left column (gated on total CD8$^+$ T cells). Their phenotypes with respect to CD62L and CD69 are shown in the next column.

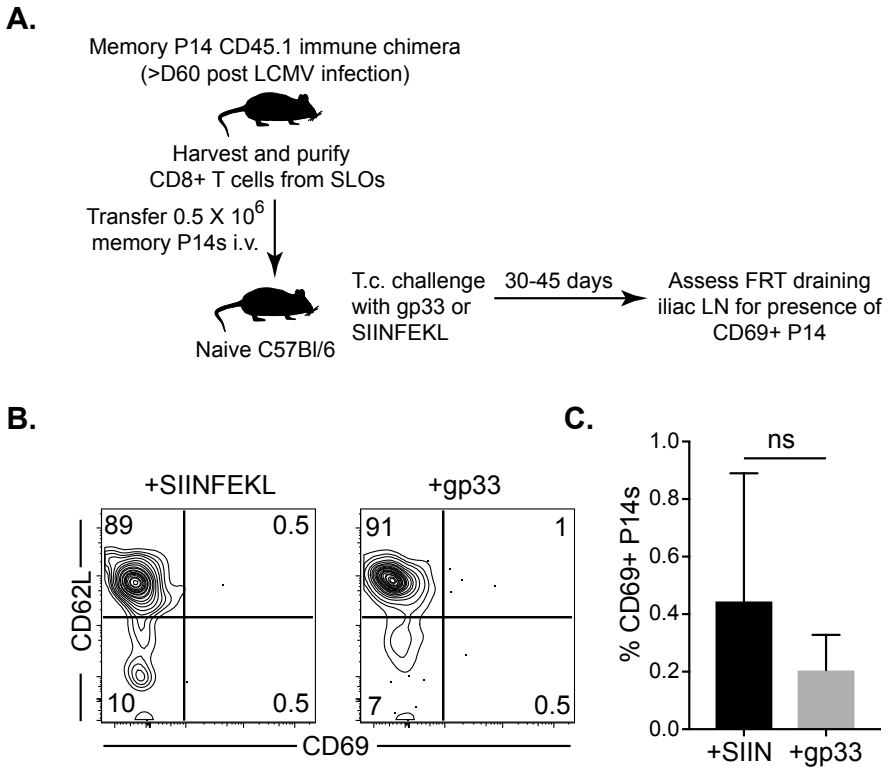

Figure S2. Related to figure 3. Transferred circulating memory CD8$^+$ T cells do not upregulate CD69 after local recall in the absence of NLT Trm cells. (A&B) CD8$^+$ T cells were purified by magnetic negative enrichment column from the pooled secondary lymphoid organs of LCMV immune chimeras, then transferred i.v. to naïve C57Bl/6 mice. Recipient mice were challenged with gp33 (or SIINFEKL control peptide) t.c. and phenotype of P14 CD8$^+$ T cells in draining LN was assessed 30-45 days later (plots gated on P14 CD8$^+$ T cells). (C) CD69 expression was at or below the limit of detection. Data are representative of two independent experiments with 3 mice/group per experiment. ns= not significant. Mann-Whitney U test (for C). Bars indicate mean ± S.E.M.

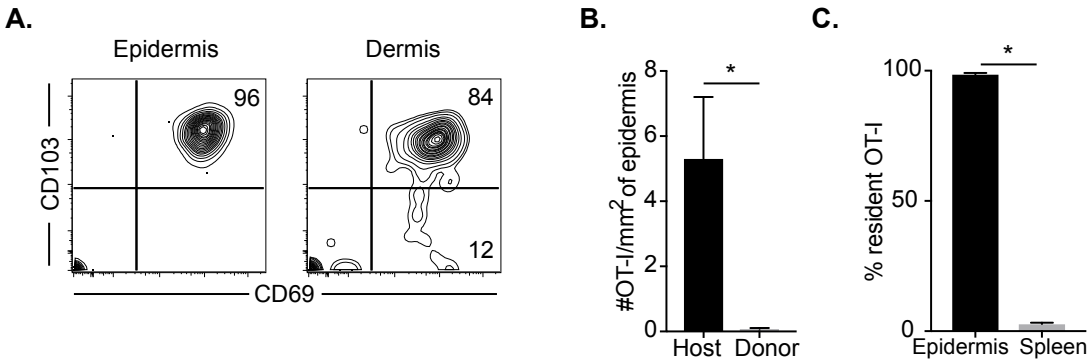

Figure S3. Related to figure 3. Systemic VSVova infection generates broadly distributed Trm cells in skin. C57Bl/6 mice received 5X10^4 OT-I CD8$^+$ T cells i.v. and were infected with 1X10^6 PFU VSVova i.v. the following day. Flank skin was harvested 60 days post-infection and epidermis and dermis were separated (see methods). (A) Phenotype of OT-I CD8$^+$ T cells isolated from epidermis or dermis. Data are representative of two independent experiments with 3 mice/group per experiment. (B-C) Memory OT-I CD90.1 and OT-I CD45.1 VSVova chimeras were surgically conjoined (parabiosis) and after confirming equilibration of OT-I (recirculating) cells in blood, OT-I CD8$^+$ T cells in the skin epidermis of donor and host origin was enumerated using quantitative immunofluorescence microscopy. Percent resident was calculated as in (Steinert et al. 2015). Data are representative of two separate experiments with 2 parabiont pairs/per experiment. * $p<0.05$. Mann-Whitney U test (for B & C). Bars indicate mean ± S.E.M.

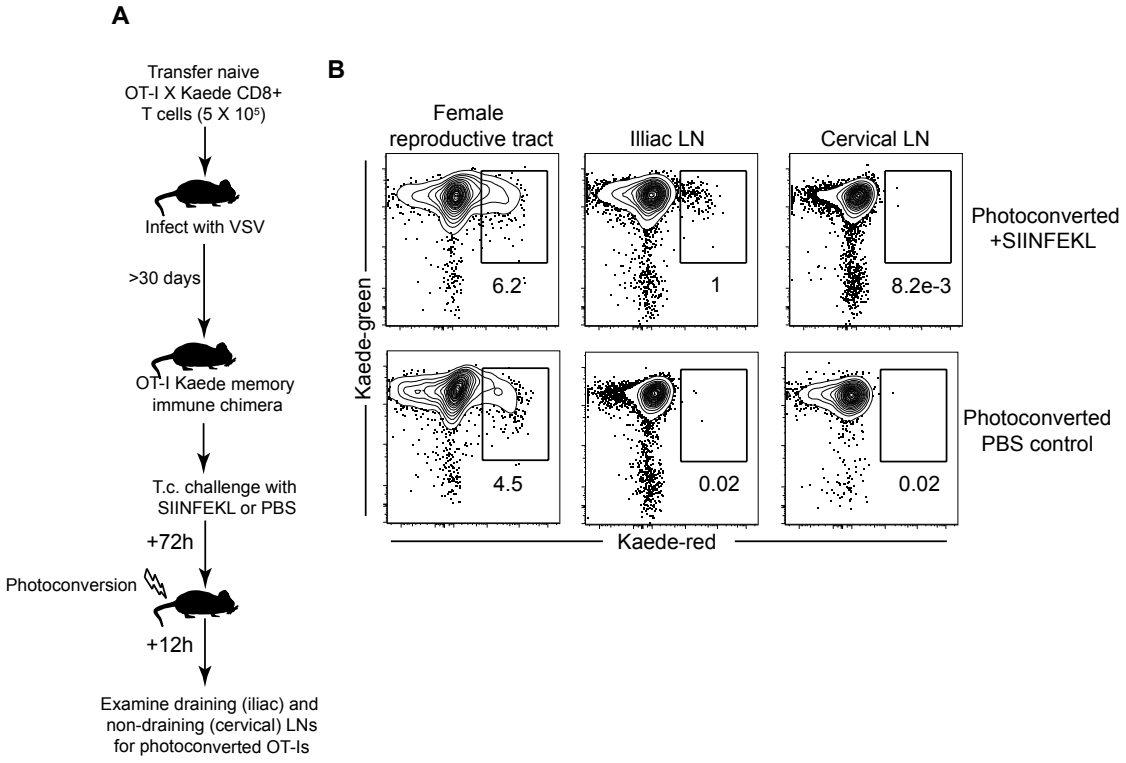

Figure S4. Related to figure 7. Reactivated FRT CD8$^+$ T cells migrate to draining LN. VSVova immune chimeric mice were made with photoactivatable OT-I CD8$^+$ T cells crossed to a KAEDE transgenic background. At least 31 days later, mice were challenged with SIINFEKL peptide t.c. as described. 72h after challenge, the uterus was exposed to violet light (wavelength=405nm) and 12h later, the indicated tissues were examined for the presence of photoconverted OT-I (Kaede-red+) CD8$^+$ T cells. Data are representative of two separate experiments with 4 mice per group per experiment.

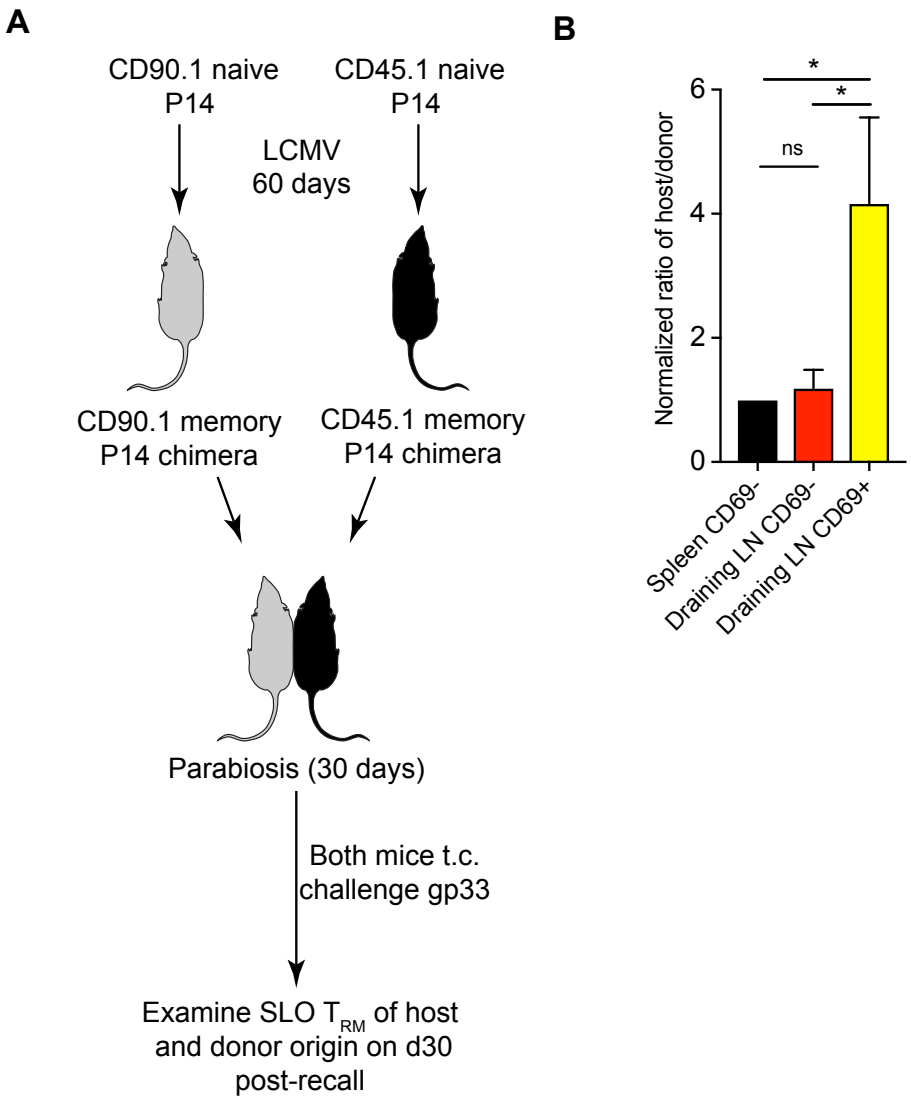

Figure S5. Related to figure 7. Resident CD8$^+$ T cells give rise to secondary SLO Trm cells. (A) Experimental design. CD45.1$^+$ and CD90.1$^+$ P14 immune chimeras were surgically conjoined by parabiosis to achieve equilibration of recirculating memory CD8$^+$ T cells. 30 days later, both mice were challenged t.c. with gp33 peptide. Spleen and draining iliac LN was analyzed by flow cytometry 30 days after t.c. challenge to evaluate host and donor P14. (B) Ratio of host to donor P14 (normalized to spleen CD69- P14) CD8$^+$ T cells 30 days post-recall is shown. Data are representative of two separate experiments with 2 parabiosis pairs/per experiment. * $p<0.05$. Kruskal-Wallis ANOVA with Dunn's multiple comparison test. Bars indicate mean ± S.E.M.